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(54) Title: ANTISENSE OLIGONUCLEOTIDE CHEMOTHERAPY FOR BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE (57) Abstract <p>Methods of selectively inhibiting the growth of or killing prostatic cells, using antisense oligonucleotides to prostate specific genes, are disclosed. The oligonucleotides may have natural nucleic acid structures or may be modified oligonucleotides with enhanced stability or tissue specific targeting. The prostate specific genes to which the antisense may be directed include the AR and the αFGF gene. Pharmaceutical compositions including such antisense oligonucleotides are also described for use in the methods. The methods and products are of particular utility in the treatment of benign prostatic hyperplasia or prostate cancer.</p>		

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ANTISENSE OLIGONUCLEOTIDE CHEMOTHERAPY FOR BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE

Field of the Invention

5 The present invention relates to the field of chemotherapy for hyperplasias and cancers and, in particular, to chemotherapy for benign hyperplasia or cancer of the prostate. In addition, the invention relates to the field of antisense oligonucleotides and their use in human hyperplasia and cancer therapy.

Background of the Invention

10 Treatment of carcinoma of the prostate was one of the first successes of cancer chemotherapy, using the therapeutic program of castration and/or anti-androgen hormonal treatments introduced by Charles Huggins in the 1940s. A remarkable relief of symptoms and objective regression of bony metastases occurs under this endocrine therapeutic program.
15 Unfortunately, after a "golden period" which lasts roughly 18 months, regrowth of the prostate cancer cells occurs and, in the later stages of the disease, sensitivity to and repression by anti-androgen hormonal therapy ceases. The conventional regimen of combined chemotherapeutic agents also is typically ineffective after the golden period, and a downhill clinical course follows, terminating in death.

20 A key problem had been the silent onset of cancer of the prostate, with growth beyond its capsule and metastasis to bone too frequently occurring before the first visit to a physician. During the last half dozen years, there has been increasing recognition of the importance of early diagnosis and significant improvements in the available tests. As a consequence of early diagnosis, detection of prostatic cancer still contained within its capsule has become more
25 frequent. For this situation, radical prostatectomy has largely supplanted the traditional castration/estrogen therapy. Radiation targeted to the prostate itself and to any proximal capsular infiltration has also become a prominent modality of therapy. When these two therapeutic approaches fail to halt progression of the disease, which is all too often (see, e.g., Gittes (1991); and Catalona (1994)), the prospect of benefit from available chemotherapy is gloomy.

30 Less severe but more common than prostatic cancer is benign prostatic hyperplasia (BPH). This condition may be a precursor to full blown prostatic cancer or may continue for decades without evolving into the deadly carcinoma. Depending upon the degree of hypertrophy

and the age of the patient, treatment may range from "watchful waiting" to more aggressive approaches employing anti-androgen hormonal therapy, transurethral resection, or radical prostatectomy (see, e.g., Catalona (1994)).

The androgen receptor (AR) binds the male hormone testosterone and, acting at the transcriptional level, regulates the growth of normal prostatic cells. A cDNA for the human AR was disclosed by Lubahn et al. (1988). As noted above, anti-androgen or estrogen hormonal therapy, including physical or chemical castration, may be effective against early stage prostate cancer but, after a period of roughly 18 months, the patient becomes refractory to the hormonal therapy. The relapse is believed to be the result of the development or clonal selection of androgen-independent tumor cells in which the AR has mutated or been lost (see, e.g., Taplin, et al. (1995); Klocker, et al. (1994). Interestingly, in murine androgen-independent prostatic cancer cells, transfection with an AR cDNA has been shown to inhibit growth in the presence of testosterone (Suzuki, et al. (1994)).

The acidic fibroblast growth factor (α FGF), also known as the heparin binding growth factor type one (HBGF-1), is an androgen-regulated mitogen produced by prostatic cells. An mRNA sequence for a human allele of α FGF was disclosed in Harris, et al. (1991). Mansson, et al. (1989) found that α FGF was expressed in normal immature rat prostate but not in normal mature rat prostate. In cancerous rat prostatic cell lines, they found α FGF expression similar to that in immature rat prostate.

Summary of the Invention

The present invention provides methods for treating a patient diagnosed as having benign prostatic hyperplasia or a prostatic cancer. The methods include administering to the patient a therapeutically effective amount of a composition comprising an antisense oligonucleotide which selectively hybridizes to an AR or α FGF gene or mRNA sequence of the patient, thereby inhibiting the expression of the AR or α FGF gene or mRNA sequence. This inhibition of the AR or α FGF genes or mRNAs by antisense oligonucleotides results in a significant inhibition of the growth or survival of prostatic cells. As a result, the methods provide a useful new means of treating benign prostatic hyperplasia and prostatic cancer. The methods are particularly useful in treating prostate cancer patients who have become refractory to anti-androgen hormonal therapy.

The AR antisense oligonucleotides may comprise at least 10 consecutive bases from SEQ

ID NO.: 1, at least 10 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 1, or oligonucleotides that hybridize to the complements of these sequences under physiological conditions. More preferably, the antisense oligonucleotides comprise at least 15 consecutive bases, and most preferably, 20-30 consecutive bases from the above-described sequences.

The α FGF antisense oligonucleotides may comprise at least 10 consecutive bases from any one of SEQ ID NO.: 2, SEQ ID NO.: 3 or SEQ ID NO.: 4, at least 10 consecutive bases from the joined exons of SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4; or oligonucleotides that hybridize to the complements of these sequences under physiological conditions. More preferably, the antisense oligonucleotides comprise at least 15 consecutive bases, and most preferably, 20-30 consecutive bases from the above-described sequences.

Examples of sequences of the invention include, but are not limited to, those disclosed as SEQ ID NO.: 5, SEQ ID NO.: 6, SEQ ID NO.: 7, and SEQ ID NO.: 8.

In preferred embodiments, all of the above-described oligonucleotides are modified oligonucleotides. In one set of embodiments, the modified oligonucleotide includes at least one synthetic internucleoside linkage such as a phosphorothioate, alkylphosphonate, phosphorodithioate, phosphate ester, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, or carboxymethyl ester.

In other embodiments with modified oligonucleotides, the modified oligonucleotide has at least one low molecular weight organic group covalently bound to a phosphate group of said oligonucleotide. In another set of embodiments, the modified oligonucleotide has at least one low molecular weight organic group covalently bound to a 2' position of a ribose of said oligonucleotide. Such low molecular weight organic groups include lower alkyl chains or aliphatic groups (e.g., methyl, ethyl, propyl, butyl), substituted alkyl and aliphatic groups (e.g., aminoethyl, aminopropyl, aminohydroxyethyl, aminohydroxypropyl), small saccharides or glycosyl groups.

In another set of embodiments the modified oligonucleotide has covalently attached thereto a prostate-targeting compound such as an androgen, androgen derivative, estrogen, estrogen derivative, estramustine, emcyt or estracyt.

In preferred embodiments, the antisense oligonucleotides are administered intravenously at a dosage between 1.0 μ g and 100 mg per kg body weight of the patient.

The present invention also provides for any or all of the above-described antisense oligonucleotides, including the various modified oligonucleotides, in a pharmaceutical composition. The antisense oligonucleotides are admixed with a sterile pharmaceutically acceptable carrier in a therapeutically effective amount such that the isolated antisense oligonucleotide selectively hybridizes to the AR or α FGF gene or mRNA sequence when administered to a patient. A pharmaceutical kit is also provided in which such a pharmaceutical composition is combined with a pharmaceutically acceptable carrier for intravenous administration.

The methods and products of the present invention further include antisense oligonucleotides, as described above, directed at a PSA gene, a probasin gene, an estrogen receptor gene, a telomerase gene, a prohibitin gene, a src gene, a ras gene, a myc gene, a bcl-2 gene, a protein kinase-A gene, a plasminogen activator urokinase gene and a methyl transferase gene.

Detailed Description of the Invention

The present invention provides new methods for the treatment of cancer of the prostate and pharmaceutical compositions useful therefor. It is now disclosed that antisense oligonucleotides complementary to genes which are expressed predominantly or strongly in prostatic cells are effective for inhibiting the growth of and/or killing hyperplastic or cancerous cells of prostatic origin. In particular, the present invention provides oligonucleotides, including modified oligonucleotides, which have antisense homology to a sufficient portion of either the AR or α FGF gene such that they inhibit the expression of that gene. Surprisingly, inhibition of either of these genes, even in androgen-resistant prostatic cancer cells, inhibits the growth of these cells. Because the antisense oligonucleotides of the invention can be administered systemically but selectively inhibit prostate cells, the present invention has particular utility in late stage prostate cancer which has metastasized.

Definitions

In order to describe more clearly and concisely the subject matter of the present invention, the following definitions are provided for specific terms used in the claims appended hereto:

AR. As used herein, the abbreviation "AR" refers to the androgen receptor well known

in the art and described in the various references cited herein. A cDNA sequence of the human AR gene was disclosed in Lubahn et al. (1988). The Lubahn et al. (1988) sequence is available on GenBank (Accession number J03180) and is reproduced here as SEQ. ID NO.: 1. The translation initiation codon of this gene is found at base positions 363-365 and the stop codon is at positions 3120-3122 of SEQ ID NO.: 1. As will be obvious to one of ordinary skill in the art, other alleles of the AR gene, including other human alleles and homologues from other mammalian species, encoding an AR protein and hybridizing to SEQ ID NO.: 1 under stringent hybridization conditions, will exist in natural populations and are embraced by the term "AR gene" as used herein.

10 α FGF. As used herein, the term " α FGF" refers to the α FGF protein known in the art and described in the various references cited herein. The genomic DNA of one allele of the human α FGF gene has been partially sequenced and was disclosed in Wang et al. (1989). The Wang et al. (1989) sequences cover the three exons of the α FGF gene as well as some 5', 3' and intron sequences. These sequences are available on GenBank (Accession numbers M23017, M23086 and M23087) and are reproduced here as SEQ. ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4. A partial cDNA sequence for a human α FGF gene also may be found in Harris et al. (1991). The locations of the exons are located in the sequence listings. The translation initiation codon is found at positions 602-604 of SEQ ID NO.: 2 and the stop codon is found at positions 496-498. In addition, as will be obvious to one of ordinary skill in the art, other alleles of the α FGF gene, including other human alleles and homologues from other mammalian species, encoding an α FGF protein and hybridizing to one or more of SEQ ID NO.: 2, SEQ ID NO.: 3 or SEQ ID NO.: 4 under stringent hybridization conditions, will exist in natural populations and are embraced by the term " α FGF gene" as used herein.

25 Antisense Oligonucleotides. As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. In particular, by an "AR-antisense oligonucleotide" and by an α FGF-antisense oligonucleotide are meant oligonucleotides which hybridize under physiological conditions to the AR gene/mRNA or α FGF gene/mRNA and, thereby, inhibit

transcription/translation of the AR and α FGF genes/mRNAs, respectively. The antisense molecules are designed so as to interfere with transcription or translation of AR or α FGF upon hybridization with the target. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be selected so as to hybridize selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions.

Stringent hybridization conditions. As used herein, the term "stringent hybridization conditions" means hybridization conditions from 30°C-60°C and from 5x to 0.1x SSC. Highly stringent hybridization conditions are at 45°C and 0.1x SSC. "Stringent hybridization conditions" is a term of art understood by those of ordinary skill in the art. For any given nucleic acid sequence, stringent hybridization conditions are those conditions of temperature and buffer solution which will permit hybridization of that nucleic acid sequence to its complementary sequence and not to substantially different sequences. The exact conditions which constitute "stringent" conditions, depend upon the length of the nucleic acid sequence and the frequency of occurrence of subsets of that sequence within other non-identical sequences. By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with identical sequences. Suitable ranges of such stringency conditions are described in Krause, M.H., and S.A. Aaronson, Methods in Enzymology, 200:546-556 (1991). As used herein with respect to in vivo hybridization conditions, the term "physiological conditions" is considered functionally equivalent to the in vitro stringent hybridization conditions.

I. Design of AR and α FGF Antisense Oligonucleotides

The present invention depends, in part, upon the discovery that the selective inhibition of the expression of AR or α FGF by antisense oligonucleotides in prostatic cells effectively inhibits cell growth and/or causes cell death.

Based upon SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4, or upon allelic or homologous genomic or cDNA sequences, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the

present invention. In order to be sufficiently selective and potent for AR or α FGF inhibition, such antisense oligonucleotides should comprise at least 10 and, more preferably, at least 15 consecutive bases which are complementary to the AR or α FGF mRNA transcripts. Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases.

- 5 Although oligonucleotides may be chosen which are antisense to any region of the AR or α FGF genes or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions or telomerase sites may be targeted. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if
- 10 alternative mRNA splicing occurs. In addition, the AR or α FGF antisense is, preferably, targeted to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al. (1994)) and at which proteins are not expected to bind. Finally, although, SEQ ID NO.: 1 discloses a cDNA sequence and SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4 disclose genomic DNA sequences, one of ordinary skill in the art may easily derive the genomic DNA corresponding to
- 15 the cDNA of SEQ ID NO.: 1 and may easily obtain the cDNA sequence corresponding to SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4. Thus, the present invention also provides for antisense oligonucleotides which are complementary to the genomic DNA corresponding to SEQ ID NO.: 1 and the cDNA corresponding to SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4. Similarly, antisense to allelic or homologous cDNAs and genomic DNAs are enabled without
- 20 undue experimentation.

- As will be understood by one of ordinary skill in the art, the antisense oligonucleotides of the present invention need not be perfectly complementary to the AR or α FGF genes or mRNA transcripts in order to be effective. Rather, some degree of mismatches will be acceptable if the antisense oligonucleotide is of sufficient length. In all cases, however, the oligonucleotides
- 25 should have sufficient length and complementarity so as to hybridize to an AR or α FGF transcript under physiological conditions. Preferably, of course, mismatches are absent or minimal. In addition, although it is not recommended, the antisense oligonucleotides may have one or more non-complementary sequences of bases inserted into an otherwise complementary antisense oligonucleotide sequence. Such non-complementary sequences may "loop" out of a
- 30 duplex formed by an AR or α FGF transcript and the bases flanking the non-complementary region. Therefore, the entire oligonucleotide may retain an inhibitory effect despite an

apparently low percentage of complementarity. Of particular importance in this respect is the use of self-stabilized or hairpin oligonucleotides. Such oligonucleotides, or modified oligonucleotides, have a sequence at the 5' and/or 3' end which is capable of folding over and forming a duplex with itself. The duplex region, which is preferably at least 4-6 bases joined by a loop of 3-6 bases, stabilizes the oligonucleotide against degradation. These self-stabilized oligonucleotides are easily designed by adding the inverted complement of a 5' or 3' AR or α FGF sequence to the end of the oligonucleotide (see, e.g., Table 1, SEQ ID NO.: 6 and SEQ ID NO.: 7; Tang, J.-Y., et al. (1993) Nucleic Acids Res. 21:2729-2735).

In one set of embodiments, the AR and α FGF antisense oligonucleotides of the invention may be composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5' end of one nucleotide and the 3' end of another nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside linkage. These oligonucleotides may be prepared by art recognized methods which may be carried out manually or by an automated synthesizer.

In preferred embodiments, however, the antisense oligonucleotides of the invention also may include "modified" oligonucleotides. That is, the oligonucleotides may be modified in a number of ways which do not prevent them from hybridizing to their target but which enhance their stability or targeting to prostatic cells or which otherwise enhance their therapeutic effectiveness. The term "modified oligonucleotide" as used herein describes an oligonucleotide in which (1) at least two of its nucleotides are covalently linked via a synthetic internucleoside linkage (i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide) and/or (2) a chemical group not normally associated with nucleic acids has been covalently attached to the oligonucleotide.

Preferred synthetic internucleoside linkages are phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidate, and carboxymethyl esters. Further, one or more of the 5'→3' phosphate group may be covalently joined to a low molecular weight (e.g., 15-500 Da) organic group. Such low molecular weight organic groups include lower alkyl chains or aliphatic groups (e.g., methyl, ethyl, propyl, butyl), substituted alkyl and aliphatic groups (e.g., aminoethyl, aminopropyl, aminohydroxyethyl, aminohydroxypropyl), small saccharides or glycosyl groups. Other low molecular weight organic modifications include additions to the

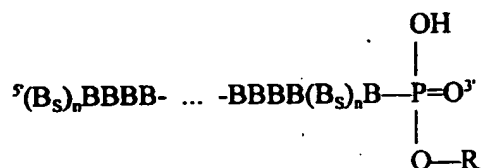
internucleoside phosphate linkages such as cholesteryl or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose. Oligonucleotides with these linkages or other modifications can be prepared according to known methods (see, e.g., Agrawal and Goodchild (1987); Agrawal et al. (1988); Uhlmann et al. (1990); Agrawal et al. (1992); Agrawal (1993); and U.S. Pat. No. 5,149,798).

The term "modified oligonucleotide" also encompasses oligonucleotides with a covalently modified base and/or sugar. For example, modified oligonucleotides include oligonucleotides having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified oligonucleotides may include a 2'-O-alkylated ribose group such as a 2'-O-methylated ribose. In addition, modified oligonucleotides may include sugars such as arabinose instead of ribose. Alternatively, the modified oligonucleotides may be branched oligonucleotides. Unoxidized or partially oxidized oligonucleotides having a substitution in one or more nonbridging oxygen per nucleotide in the molecule are also considered to be modified oligonucleotides.

Also considered as modified oligonucleotides are oligonucleotides having prostate-targeting, nuclease resistance-conferring, or other bulky substituents and/or various other structural modifications not found *in vivo* without human intervention. The androgen receptor and other hormonal receptor sites on prostate cells allow for targeting antisense oligonucleotides specifically or particularly to prostatic cells. Attachment of the antisense oligonucleotides by a molecular "tether" (e.g., an alkyl chain) to estramustine, emcyt or estracyt (Sheridan and Tew (1991)), for example, may provide prostatic targeting and the possibility of covalent alkylation of host prostatic DNA. Estramustine targets particularly to the ventral prostate (Forsgren, et al. (1979)). Similarly, one may covalently attach androgen, estrogen, androgen or estrogen derivatives, or other prostate cell ligands to antisense oligonucleotides using tethers and conjugating linkages for prostatic targeting. Finally, one may of course covalently attach other chemotherapeutic agents (e.g., dexamethasone, vinblastine, etoposide) to the antisense oligonucleotides for enhanced effect.

The most preferred modified oligonucleotides are hybrid or chimeric oligonucleotides in which some but not all of the phosphodiester linkages, bases or sugars have been modified. Hybrid modified antisense oligonucleotides may be composed, for example, of stretches of ten

- 2'-O-alkyl nucleotides or ten phosphorothioate synthetic linkages at the 5' and/or 3' ends, and a segment of seven unmodified oligodeoxynucleotides in the center, or of similar terminal segments of alkyl phosphonates, with central P=S or P=O oligonucleotides (Agrawal, et al. (1990); Metelev, et al. (1994)). The currently most preferred modified oligonucleotides are 2'-O-methylated hybrid oligonucleotides. Since degradation occurs mainly at the 3' end, secondarily at the 5' end, and less in the middle, unmodified oligonucleotides located at this position can activate RNase H, and yet are degraded slowly. Furthermore, the T_m of such a 27-mer is approximately 20°C higher than that of a 27-mer all phosphorothioate oligodeoxynucleotide. This greater affinity for the targeted genomic area can result in greater inhibiting efficacy.
- Obviously, the number of synthetic linkages at the termini need not be ten and synthetic linkages may be combined with other modifications, such as alkylation of a 5' or 3' phosphate, or 2'-O-alkylation. Thus, merely as another example, one may produce a modified oligonucleotide with the following structure, where B represents any base, R is an alkyl, aliphatic or other substituent, the subscript S represents a synthetic (e.g. phosphorothioate) linkage, and each n is an independently chosen integer from 1 to about 20:



II. Products and Methods of Treatment for BPH and Prostate Cancer

The methods of the present invention represent new and useful additions to the field of benign prostate hyperplasia or prostate cancer therapy. In particular, the methods of the present invention are especially useful for late stage prostate cancer in which metastases have occurred and in which the cells have become resistant to estrogen or anti-androgen therapy. The methods may, however, also be used in benign prostate hyperplasia or early stage prostate cancer and may provide a substitute for more radical procedures such as transurethral resection, radical prostatectomy, or physical or chemical castration. The products of the present invention include the isolated antisense oligonucleotides described above. As used herein, the term "isolated" as applied to an antisense oligonucleotide means not covalently bound to and physically separated from the 5' and 3' sequences which flank the corresponding antisense sequence in nature.

Administration of the AR or α FGF antisense oligonucleotides may be oral, intravenous,

parenteral, cutaneous or subcutaneous. For BPH or when the site of a prostatic tumor is known, the administration also may be localized to the prostate or to the region of the tumor by injection to or perfusion of the site.

AR or α FGF antisense oligonucleotides may be administered as part of a pharmaceutical composition. Such a pharmaceutical composition may include the antisense oligonucleotides in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain a therapeutically effective amount of the antisense oligonucleotides in a unit of weight or volume suitable for administration to a patient. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Physiologically and pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art. The pharmaceutical composition of the invention may also contain other active factors and/or agents which inhibit prostate cell growth or increase cell death. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect or to minimize side-effects caused.

The pharmaceutical composition of the invention may be in the form of a liposome in which the AR or α FGF antisense oligonucleotides are combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which are in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. No. 4,235,871; U.S. Pat. No. 4,501,728; U.S. Pat. No. 4,837,028; and U.S. Pat. No. 4,737,323.

The pharmaceutical composition of the invention may further include compounds such as cyclodextrins and the like which enhance delivery of oligonucleotides into cells. When the composition is not administered systemically but, rather, is injected at the site of the target cells, cationic detergents (e.g. Lipofectin) may be added to enhance uptake.

When a therapeutically effective amount of AR or α FGF antisense oligonucleotides is administered orally, the oligonucleotides will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder may contain from about 5 to 95% of the AR and/or α FGF antisense oligonucleotides and preferably from about 25 to 90% of the oligonucleotides. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition may contain from about 0.5 to 90% by weight of an AR and/or α FGF antisense oligonucleotide and preferably from about 1 to 50% of the oligonucleotide.

When a therapeutically effective amount of an AR or α FGF antisense oligonucleotide is administered by intravenous, cutaneous or subcutaneous injection, the oligonucleotides will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to the antisense oligonucleotides, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or another vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

In preferred embodiments, when the target cells are readily accessible, administration of the antisense oligonucleotides is localized to the region of the targeted cells in order to maximize the delivery of the antisense and to minimize the amount of antisense needed per treatment. Thus, in one preferred embodiment, administration is by direct injection at or perfusion of the site of the targeted cells, such as a tumor. Alternatively, the antisense oligonucleotides may be adhered to small particles (e.g., microscopic gold beads) which are impelled through the membranes of the target cells (see, e.g., U.S. Pat. No. 5,149,655).

In another series of embodiments, a recombinant gene is constructed which encodes an

AR or α FGF antisense oligonucleotide and this gene is introduced within the targeted cells on a vector. Such an AR or α FGF antisense gene may, for example, consist of the normal AR or α FGF sequence, or a subset of the normal sequences, operably joined in reverse orientation to a promoter region. An operable antisense gene may be introduced on an integration vector or may
5 be introduced on an expression vector. In order to be most effective, it is preferred that the antisense sequences be operably joined to a strong eukaryotic promoter which is inducible or constitutively expressed.

In all of the above-described methods of treatment, the AR and/or α FGF antisense oligonucleotides are administered in therapeutically effective amounts. As used herein, the term
10 "therapeutically effective amount" means that amount of antisense which, under the conditions of administration, including mode of administration and presence of other active components, is sufficient to result in a meaningful patient benefit, i.e., the killing or inhibition of the growth of target cells.

The amount of AR and/or α FGF antisense oligonucleotides in the pharmaceutical
15 composition of the present invention will depend not only upon the potency of the antisense but also upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of antisense with which to treat each individual patient. Initially, the attending physician will administer low doses of the inhibitor and observe the patient's response. Larger doses of
20 antisense may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. In preferred embodiments, it is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 1.0 μ g to about 100 mg of oligonucleotide per kg body weight.

The duration of intravenous therapy using the pharmaceutical compositions of the present
25 invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. Because a bolus of oligonucleotides, particularly highly negatively-charged phosphorothioate modified oligonucleotides, may have adverse side effects (e.g., rapid lowering of blood pressure), slow intravenous administration is preferred. Thus, intravenous administration of therapeutically effective amounts over a 12-24
30 hour period are contemplated. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

The following examples of the use of AR and α FGF antisense are presented merely to illustrate some of the oligonucleotides, including modified oligonucleotides, that may be employed according to the present invention. The particular oligonucleotides used, therefore, should not be construed as limiting of the invention but, rather, as indicative of the wide range of oligonucleotides which may be employed. As will be obvious to one of ordinary skill in the art in light of the present disclosure, a great many equivalents to the presently disclosed antisense oligonucleotides and disclosed methods are now available. In particular, other antisense oligonucleotides substantially complementary to subsets of SEQ ID NO.: 1, SEQ ID NO.: 2, SEQ ID NO.: 3 or SEQ ID NO.: 4 and chemical modifications of the same which do not prevent hybridization under physiological conditions, are contemplated as equivalents of the examples presented below. In general, the use of prostate specific antisense oligonucleotides is contemplated as a method of selectively inhibiting the growth of or killing prostatic cells. In particular, the use of antisense oligonucleotides to the estrogen receptor, PSA, probasin, telomerase, prohibitin, src, ras, myc, bcl-2, protein kinase-A, plasminogen activator urokinase and methyl transferase genes is contemplated for the treatment of benign prostatic hyperplasia or prostatic cancer.

Experimental Examples

The PC3-1435 permanent cell line of human prostatic cancer, obtained from the American Type Culture Collection, was grown in monolayer culture: The PC3-1435 cells are from an osseous metastasis and are androgen-insensitive. Cells were grown in Dulbecco's medium supplemented with 10 percent fetal calf serum, glutamate, pyruvate, penicillin and streptomycin, in 25-150 cm flasks, incubated at 37°C in 6 percent CO₂-air.

A number of AR and α FGF antisense oligonucleotides were tested for their inhibitory effect on prostatic cells. The base sequences of these oligonucleotides are disclosed as SEQ ID NO.: 5 through SEQ ID NO.: 8. SEQ ID NO.: 5 is antisense to positions 927-953 of the AR gene (SEQ ID NO.: 1). SEQ ID NO.: 6 is a self-stabilized or hairpin oligonucleotide. The first 21 bases are complementary to positions 916-936 of the AR gene. The remaining eight are identical to positions 920-927 of the gene, allowing formation of a 3' hairpin. SEQ ID NO.: 7 is another self-stabilized antisense oligonucleotide. The first 21 bases of this oligonucleotide are complementary to positions 927-947 of the AR gene. The remaining eight are identical to

positions 931-938 of the gene, allowing for formation of a 3' hairpin. Finally, SEQ ID NO.: 8 is an antisense sequence corresponding to positions 611-635 of the α FGF gene.

Table 1 shows some of the antisense oligonucleotides tested. The numbers at the left of each sequence correspond to the sequence numbers in the sequence listing. Antisense oligonucleotides with unmodified or natural internucleoside linkages (P=O) and oligonucleotides with all phosphorothioate synthetic linkages (P=S) were tested. In addition, modified oligonucleotides were tested in which just the terminal two phosphodiester linkages at each end had been replaced by phosphorothioate synthetic linkages (shown as a subscript S between nucleotides in Table 1) and/or in which small organic chemical groups (e.g., 2-hydroxy-3-amino-propyl, propylamine) were added to the 3' terminal phosphate or the penultimate 3' phosphate.

Growth of the PC3-1435 cell line in tissue culture monolayers was consistently inhibited by addition of phosphorothioate-modified oligodeoxynucleotides targeted against the AR or α FGF genes and incubation for 24-48 hours thereafter. As the concentration of modified oligonucleotides is decreased from the 10-20 μ M level, most effective inhibition occurs with specific antisense oligodeoxynucleotides at the 2-5 μ M level, as contrasted with mismatched oligodeoxynucleotides (see Tables 2 and 3).

While the effects on cell growth (i.e. cell numbers) are readily manifest, visual substage microscopy of wells revealed additional features of the inhibition events using AR antisense oligonucleotides against PC3-1435 cells. The first evidence of antisense inhibition is rupture of the monolayer fabric. The stellate cells in a confluent culture lose contact with their neighbors, round up individually or in clumps, become pyknotic, and cease growing, as examined on successive days. There is an early loss of adhesiveness to the floor of the plastic wells. These changes are more severe (see Table 4) than those measured by 3 H-thymidine incorporation into DNA, in other words more drastic than the impairment of DNA synthesis.

Each of the above-mentioned references and patents are incorporated by reference.

TABLE 1
Antisense Oligonucleotides

	Sequence	Target
5		
	#5 5'CTG-CTG-CTG-TTG-CTG-AAG-GAG-TTG-CAT ^{3'}	Androgen
	receptor,	
		P=S
	#5 5'CTG-CTG-CTG-TTG-CTG-AAG-GAG-TTG-CAT ^{3'}	Androgen
10	receptor,	
		P=O
	#5 5'C _s T _s G-CTG-CTG-TTG-CTG-AAG-GAG-TTG-C _s A _s T ^{3'}	Androgen
	receptor,	
		P=S termini
15	#5 5'CTG-CTG-CTG-TTG-CTG-AAG-GAG-TTG-CAT ^{3'}	Androgen
	receptor,	
		modified with
		organic group
20	$ \begin{array}{c} + \\ \text{H}_3\text{N}-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{O}-\text{P}(=\text{O})(\text{OH})_2 \end{array} $	
25	receptor,	Androgen
	#5 5'CTG-CTG-CTG-TTG-CTG-AAG-GAG-TTG-CA-O-P(=O)(OH)-O-T ^{3'}	modified with
		organic group
	CH ₃ CH ₂ CH ₂ NH	
30		
	#6 5'GGA-GTT-GCA-TGG-TGC-TGG-CCT-CAG-CAC-CA ^{3'}	Androgen
	receptor	
		3' hairpin, P=S
	#7 5'CTG-TTG-CTG-AAG-GAG-TTG-CAT-AAC-TCC-TT ^{3'}	Androgen
35	receptor	
		3' hairpin, P=S
	#8 5'GGG-CTG-TGA-AGG-TGG-TGA-TTT-CCC-C ^{3'}	αFGF, P=S

#8 5'GGG-CTG-TGA-AGG-TGG-TGA-TTT-CCC-C^{3'} α FGF, P=O

TABLE 2

³H-thymidine incorporation into DNA PC3-1435

human prostate cancer tissue culture

Genes Targeted	Concentration (μ M)	CPM†	% inhibition
Control (no oligo)	--	38,000	0
Androgen receptor, (P = S)	20	15,000	60
	5	20,000	48
Androgen receptor, (P = S)*	20	10,200	68
	5	24,000	25
Mismatch (P = S)	20	20,000	47
	5	27,000	30

† Averages of 3 separate wells

* 3' phosphate modified with -CH₂CHOHCHNH₃⁺

TABLE 3

Degree of inhibition of DNA synthesis
in PC3-1435 prostate cancer tissue cultures

Genes targeted	Concentration (μ M)	CPM †	% inhibition
Control (no oligo)	--	14,700	0
α FGF (P=S)	20	2,485	83
	5	4,500	69
Mismatch	20	6,990	51
	5	10,750	27

† Averages of 3 separate wells.

TABLE 4

Morphological Comparison of Treated and Control Cells

5

	<u>Gene Target</u>	<u>Concentration μM</u>			
		20	10	5	2
10	α FGF (P=S)	4+	4+	1-1/2+	1+
	Androgen receptor (P=S)	3+	3+	1+	1+
	Mismatch (P=S)	1-1/2+	1/2+	0	0

Observation 24 hours after oligonucleotide addition. Damage: 4+ devastating; 3+ severe; 2+ serious; 1+ visible; 1/2+ slight; 0 none

15

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT: WORCESTER FOUNDATION FOR BIOMEDICAL RESEARCH, INC.

(ii) TITLE OF INVENTION: ANTISENSE OLIGONUCLEOTIDE CHEMOTHERAPY
FOR BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE

10

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: WOLF, GREENFIELD & SACKS, P.C.

15

(B) STREET: 600 ATLANTIC AVENUE

(C) CITY: BOSTON

(D) STATE: MA

(E) COUNTRY: USA

(F) ZIP: 02210

20

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

25

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

30

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: TWOMEY, MICHAEL J.
- (B) REGISTRATION NUMBER: 38,349
- (C) REFERENCE/DOCKET NUMBER: W0461/7035

5

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 617-720-3500
- (B) TELEFAX: 617-720-2441

10

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3569 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: cDNA

20

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 363..3122

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

35 TAATAACTCA GTTCTTATTT GCACCTACTT CAGTGGACAC TGAATTTGGA AGGTGGAGGA 60

TTTTGTTTTT TTCTTTTAAG ATCTGGGCAT CTTTGAATC TACCCTTCAA GTATTAAGAG 120

ACAGACTGTG AGCCTAGCAG GGCAGATCTT GTCCACCGTG TGTCTTCTTC TGCACGAGAC 180

5 TTTGAGGCTG TCAGAGCGCT TTTTGCCTGG TTGCTCCCGC AAGTTTCCTT CTCTGGAGCT 240

TCCCGCAGGT GGGCAGCTAG CTGCAGCGAC TACCGCATCA TCACAGCCTG TTGAACTCTT 300

CTGAGCAAGA GAAGGGGAGG CGGGGTAAGG GAAGTAGGTG GAAGATTCAG CCAAGCTCAA 360

10

GG ATG GAA GTG CAG TTA GGG CTG GGA AGG GTC TAC CCT CGG CCG CCG 407

Met Glu Val Gln Leu Gly Leu Gly Arg Val Tyr Pro Arg Pro Pro

1

5

10

15

15 TCC AAG ACC TAC CGA GGA GCT TTC CAG AAT CTG TTC CAG AGC GTG CGC 455

Ser Lys Thr Tyr Arg Gly Ala Phe Gln Asn Leu Phe Gln Ser Val Arg

20

25

30

GAA GTG ATC CAG AAC CCG GGC CCC AGG CAC CCA GAG GCC GCG AGC GCA 503

20 Glu Val Ile Gln Asn Pro Gly Pro Arg His Pro Glu Ala Ala Ser Ala

35

40

45

GCA CCT CCC GGC GCC AGT TTG CTG CTG CTG CAG CAG CAG CAG CAG CAG 551

Ala Pro Pro Gly Ala Ser Leu Leu Leu Leu Gln Gln Gln Gln Gln Gln

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	CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAA GAG	599
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	65 70 75	
5	ACT AGC CCC AGG CAG CAG CAG CAG CAG CAG GGT GAG GAT GGT TCT CCC	647
	Thr Ser Pro Arg Gln Gln Gln Gln Gln Gln Gly Glu Asp Gly Ser Pro	
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	CAA GCC CAT CGT AGA GGC CCC ACA GGC TAC CTG GTC CTG GAT GAG GAA	695
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	100 105 110	
	CAG CAA CCT TCA CAG CCG CAG TCG GCC CTG GAG TGC CAC CCC GAG AGA	743
	Gln Gln Pro Ser Gln Pro Gln Ser Ala Leu Glu Cys His Pro Glu Arg	
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	GGT TGC GTC CCA GAG CCT GGA GCC GCC GTG GCC GCC AGC AAG GGG CTG	791
	Gly Cys Val Pro Glu Pro Gly Ala Ala Val Ala Ala Ser Lys Gly Leu	
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	145 150 155	
25	TCC ACG TTG TCC CTG CTG GGC CCC ACT TTC CCC GGC TTA AGC AGC TGC	887
	Ser Thr Leu Ser Leu Leu Gly Pro Thr Phe Pro Gly Leu Ser Ser Cys	
	160 165 170 175	
	TCC GCT GAC CTT AAA GAC ATC CTG AGC GAG GCC AGC ACC ATG CAA CTC	935
30	Ser Ala Asp Leu Lys Asp Ile Leu Ser Glu Ala Ser Thr Met Gln Leu	
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	CTT CAG CAA CAG CAG CAG GAA GCA GTA TCC GAA GGC AGC AGC AGC GGG	983
	Leu Gln Gln Gln Gln Gln Glu Ala Val Ser Glu Gly Ser Ser Ser Gly	
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	Arg Ala Arg Glu Ala Ser Gly Ala Pro Thr Ser Ser Lys Asp Asn Tyr	
	210 215 220	
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	Leu Gly Gly Thr Ser Thr Ile Ser Asp Asn Ala Lys Glu Leu Cys Lys	
	225 230 235	
	GCA GTG TCG GTG TCC ATG GGC CTG GGT GTG GAG GCG TTG GAG CAT CTG	1127
10	Ala Val Ser Val Ser Met Gly Leu Gly Val Glu Ala Leu Glu His Leu	
	240 245 250 255	
	AGT CCA GGG GAA CAG CTT CGG GGG GAT TGC ATG TAC GCC CCA CTT TTG	1175
	Ser Pro Gly Glu Gln Leu Arg Gly Asp Cys Met Tyr Ala Pro Leu Leu	
15	260 265 270	
	GGA GTT CCA CCC GCT GTG CGT CCC ACT CCT TGT GCC CCA TTG GCC GAA	1223
	Gly Val Pro Pro Ala Val Arg Pro Thr Pro Cys Ala Pro Leu Ala Glu	
	275 280 285	
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	290 295 300	
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	GGC GAG AGC CTA GGC TGC TCT GGC AGC GCT GCA GCA GGG AGC TCC GGG	1367
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	320 325 330 335	
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5 GCT CTG GCC GGA CCG CCG CCC CCT CCG CCG CCT CCC CAT CCC CAC GCT 1511
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 10 Arg Ile Lys Leu Glu Asn Pro Leu Asp Tyr Gly Ser Ala Trp Ala Ala
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 Pro Tyr Pro Ser Pro Thr Cys Val Lys Ser Glu Met Gly Pro Trp Met
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GAT AGC TAC TCC GGA CCT TAC GGG GAC ATG CGT TTG GAG ACT GCC AGG 1991
 10 Asp Ser Tyr Ser Gly Pro Tyr Gly Asp Met Arg Leu Glu Thr Ala Arg
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 Asp His Val Leu Pro Ile Asp Tyr Tyr Phe Pro Pro Gln Lys Thr Cys
 15 545 550 555

CTG ATC TGT GGA GAT GAA GCT TCT GGG TGT CAC TAT GGA GCT CTC ACA 2087
 Leu Ile Cys Gly Asp Glu Ala Ser Gly Cys His Tyr Gly Ala Leu Thr
 560 565 570 575

20 TGT GGA AGC TGC AAG GTC TTC TTC AAA AGA GCC GCT GAA GGG AAA CAG 2135
 Cys Gly Ser Cys Lys Val Phe Phe Lys Arg Ala Ala Glu Gly Lys Gln
 580 585 590

25 AAG TAC CTG TGC GCC AGC AGA AAT GAT TGC ACT ATT GAT AAA TTC CGA 2183
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 595 600 605

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	CGA TCC TTC ACC AAT GTC AAC TCC AGG ATG CTC TAC TTC GCC CCT GAT	2663
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	755 760 765	
	CTG GTT TTC AAT GAG TAC CGC ATG CAC AAG TCC CGG ATG TAC AGC CAG	2711
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 785 790 795

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 820 825 830

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 15 835 840 845

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 850 855 860

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GCA GAG ATC ATC TCT GTG CAA GTG CCC AAG ATC CTT TCT GGG AAA GTC 3095
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AAG CCC ATC TAT TTC CAC ACC CAG TGAAGCATTG GAAACCCTAT TTCCCCACCC 3149
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 35 915 920

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TTTAGAGAGC TAAGATTATC TGGGGAAATC AAAACAAAAA ACAAGCAAAC AAAAAAAAAA 3569

15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1082 base pairs

20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

25

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

30

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

(A) NAME/KEY: exon

35

(B) LOCATION: 602..770

(D) OTHER INFORMATION: /note= "SEGMENT 1 OF 3."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AAGCTTCCCT TAACATACTA ACCCTTTACT TTCCCTGTTG TGTCCCTGAA AGGCCTCCTG 60

5 TGCCTTTGGC TGCAGGTCCC GAACGTCCAG GCCATCTGTG CTATCTGCTT CGCGGTACCT 120

CACCAACGCA ACGTGAGGGT GGAGGGCAGA ACCTTGGTCC TGGCCTCTCA GCTTTTGTGG 180

GTTTCAGCCA GACCCTAGGT GTTATTTTAG TGCAACTTTG GTGTTTAATT TGAGGATGTG 240

10 TGTGGACCAG AAGGAGGGAC CAAAACATGA TTCTTTTCCC CATGGTCAGA TGATTAAATT 300

TGAAGTTCTA AAAAATGCAG TTTGGTCCAA AGCTGTGTCC AATTGGGAAG AGAGAAAAAT 360

15 GCCCTGGAAA CCCCTCCCAG GCCTGGGACC ATCCTTCCTT AACCACCAGC CACCTCACAG 420

GCCCGCGGAC TGCGGGCATC ACCTGGGCAG GCTGTGCTTA CTCACTACCC GGAACCCTG 480

TGCCCTGGAG CTGTCCTTCC TCTCTTCAAA GTGCATTTTG TGCCTTTGCT GGAAGAACCG 540

20 ACTACAGGTT TGTTC AATTT CTTACAGTCT TGAAAGCGCC ACAAGCAGCA GCTGCTGAGC 600

CATGGCTGAA GGGGAAATCA CCACCTTCAC AGCCCTGACC GAGAAGTTTA ATCTGCCTCC 660

25 AGGGAATTAC AAGAAGCCCA AACTCCTCTA CTGTAGCAAC GGGGGCCACT TCCTGAGGAT 720

CCTTCCGGAT GGCACAGTGG ATGGGACAAG GGACAGGAGC GACCAGCACA GTAAGCCCAT 780

CTCTATGGCA CCCCCCTTCC CTTTCTGACA TCTTCTGTAG TCAAGGTGGG AGGAAGGTGC 840

30 ACATTTAAGT ACAGGTACTT GCTTCTCCAA GGTTCATTTC AGGCATGACA CATTGAGAGG 900

TGGAGTCACA TAAATGCGTA AAATGTCTGG GAAATGAAAA TAGGGACTTG TGGGGGCCAC 960

35 CACTTACCCA AACGTGTCCT ATTTCAAGTT TTTTAAAGCA CTCTCTGCTG ACCCAACAGA 1020

ACGGGCTGCC GGTGCTCAAT TGCTGTATGT TTTCCCAGGT TTCTGTA ACT AGTGAAAGAT 1080

CT

1082

5 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 427 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

20 (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 186..289

25 (D) OTHER INFORMATION: /note= "SEGMENT 2 OF 3. UNKNOWN
NUMBER OF BP AFTER SEGMENT 1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30

CAGCTTTCTT TGGAAGGCAA AGAAAAAGGG ACTGTATTTT TATGTTTTGA TTAATCTGAG 60

GCTCATCCTG AGGGCTCCGT GAAATGAATG AGCAGAATTT TCCATGGCCA ACTGTCCTGG 120

35 CTGCCGGGTC CTATCGGCAA AAGCGTAGTG TTTATTTACT TTTGCTCGTG TTATTTTTAT 180

TCCAGTTCAG CTGCAGCTCA GTGCGGAAAG CGTGGGGGAG GTGTATATAA AGAGTACCGA 240

GACTGGCCAG TACTTGGCCA TGGACACCGA CGGGCTTTTA TACGGCTCAG TAAGTATGAA 300

5 GCTGACATGC TTCCAGACGT TGGCCAAGGT TTGAGGTTTC CAGAAATCTT GTTACATGGA 360

GTGAGGCAAA CTATAAAGCA ACAATTAGTC TCTGTTTGTT ATTTTITCCA GAAGGATTCC 420

CACCCTC 427

10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 664 base pairs

15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

(A) NAME/KEY: exon

30

(B) LOCATION: 304..498

(D) OTHER INFORMATION: /note= "SEGMENT 3 OF 3. UNKNOWN

NUMBER OF BP AFTER SEGMENT 2."

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGAGGACTCT TAGAAGTGCT CTTATCAGTA GCATCTTAAT TACTTTACAA TGGATTTTAA 60
ATGGAAAGGA AGTTTACAAT AATAGCAAAT GCATATTGAC AGCTCTTTAG TGCCCGGTGC 120
5 TGTTCCTAAGT CCTTATGACT ACCCTGTGAA ATAAGTTCCA CCATGACCCC AATTTTCCTG 180
AAAAGGAGAC TGAGGCATGG AGAGCTTTAG TATTTTGCCC AATGTCACAC AGCTAGTAAA 240
TGGGGACCCC CATGTGAAAC TACTCACTGA TTGTCCTACT CTCTGTGGT TTTATCTTTT 300
10 TAGCAGACAC CAAATGAGGA ATGTTTGTTT CTGGAAAGGC TGGAGGAGAA CCATTACAAC 360
ACCTATATAT CCAAGAAGCA TGCAGAGAAG AATTGGTTTG TTGGCCTCAA GAAGAATGGG 420
15 AGCTGCAAAC GCGGTCCTCG GACTCACTAT GGCCAGAAAG CAATCTTGT TCTCCCCCTG 480
CCAGTCTCTT CTGATTAAAG AGATCTGTTT TGGGTGTTGA CCACTCCAGA GAAGTTTCGA 540
GGGGTCCTCA CCTGGTTGAC CCAAAAATGT TCCCTTGACC ATTGGCTGCG CTAACCCCCA 600
20 GCCCACAGAG CCTGAATTTG TAAGCAACTT GCTTCTAAAT GCCCAGTTCA CTTCTTTGCA 660
GAGC 664

25 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
30 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

5

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..27

(D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS

10

927-953 OF SEQ ID NO.: 1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

15 CTGCTGCTGT TGCTGAAGGA GTTGCAT

27

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

30

(vi) ORIGINAL SOURCE:

(A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

(ix) FEATURE:

35

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..21

(D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS
916-936 OF SEQ ID NO.: 1."

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAGTTGCAT GGTGCTGGCC TCAGCACCA

29

10 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

25 (A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..21

30 (D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS
927-947 OF SEQ ID NO.: 1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

35

CTGTTGCTGA AGGAGTTGCA TAACTCCTT

29

(2) INFORMATION FOR SEQ ID NO:8:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

15

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

20

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..25
- (D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS
611-635 OF SEQ ID NO.: 2."

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGGCTGTGAA GGTGGTGATT TCCCC

25

30

CLAIMS

We claim:

1. A method for treating a patient diagnosed as having benign prostatic hyperplasia
5 or a prostatic cancer comprising
administering to said patient a therapeutically effective amount of a composition
comprising an antisense oligonucleotide which selectively hybridizes to a gene or mRNA
sequence of said patient;
wherein said antisense inhibits expression of said gene or mRNA sequence; and
10 wherein said gene or mRNA sequence is selected from the group consisting of an AR and
an α FGF gene or mRNA sequence.
2. A method as in claim 1 wherein said oligonucleotide is selected from the group
consisting of
15 (a) oligonucleotides comprising at least 10 consecutive bases from SEQ ID NO.: 1;
(b) oligonucleotides comprising at least 10 consecutive bases from a genomic sequence
corresponding to SEQ ID NO.: 1; and
(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or
(b) under physiological conditions.
20
3. A method as in claim 1 wherein said oligonucleotide is selected from the group
consisting of
(a) oligonucleotides comprising at least 20 consecutive bases from SEQ ID NO.: 1;
(b) oligonucleotides comprising at least 10 consecutive bases from a genomic sequence
25 corresponding to SEQ ID NO.: 1; and
(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or
(b) under physiological conditions.

4. A method as in claim 1 wherein said oligonucleotide is selected from the group consisting of

(a) oligonucleotides comprising at least 10 consecutive bases from the group consisting of SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4;

5 (b) oligonucleotides comprising at least 10 consecutive bases from the joined exons of SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4; and

(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or (b) under physiological conditions.

10 5. A method as in claim 1 wherein said oligonucleotide is selected from the group consisting of

(a) oligonucleotides comprising at least 20 consecutive bases from the group consisting of SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4;

(b) oligonucleotides comprising at least 20 consecutive bases from the joined exons of
15 SEQ ID NO.: 2, SEQ ID NO.: 3 and SEQ ID NO.: 4; and

(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or (b) under physiological conditions.

6. A method as in claim 1 wherein said oligonucleotide comprises a nucleotide
20 sequence selected from the group consisting of SEQ ID NO.: 5, SEQ ID NO.: 6, SEQ ID NO.: 7, and SEQ ID NO.: 8.

7. A method as in claim 1 wherein said oligonucleotide is a modified oligonucleotide.

25

8. A method as in claim 7 wherein said oligonucleotide is a modified oligonucleotide including at least one synthetic internucleoside linkage.

9. A method as in claim 8 wherein said synthetic internucleoside linkage is selected
30 from the group consisting of phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate

triesters, acetamidates, and carboxymethyl esters.

10. A method as in claim 7 wherein said oligonucleotide is a modified
oligonucleotide having at least one low molecular weight organic group covalently bound to a
5 phosphate group of said oligonucleotide.

11. A method as in claim 7 wherein said oligonucleotide is a modified
oligonucleotide having at least one low molecular weight organic group covalently bound to a 2'
position of a ribose of said oligonucleotide.
10

12. A method as in claim 7 wherein said oligonucleotide is a modified
oligonucleotide having covalently attached thereto a compound selected from the group
consisting of androgen, androgen derivatives, estrogen, estrogen derivatives, estramustine, emcyt
and estracyt.
15

13. A method as in claim 1 wherein said oligonucleotide is administered
intravenously at a dosage between 1.0 μ g and 100 mg per kg body weight of said patient.

14. A method as in claim 1 wherein said patient has a prostatic cancer which is
20 refractory to anti-androgen or estrogen hormonal therapy.

15. A pharmaceutical composition comprising
a sterile pharmaceutically acceptable carrier; and
a therapeutically effective amount of an isolated antisense oligonucleotide which
25 selectively hybridizes to a gene or mRNA sequence of a patient;
wherein said antisense inhibits expression of said gene or mRNA sequence; and
wherein said gene or mRNA sequence is selected from the group consisting of an AR and
an α FGF gene or mRNA sequence.

30 16. A composition as in claim 15 wherein said oligonucleotide is selected from the
group consisting of

- (a) oligonucleotides comprising at least 10 consecutive bases from SEQ ID NO.: 1;
- (b) oligonucleotides comprising at least 10 consecutive bases from the joined exons of SEQ ID NO.: 1; and

- (c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or
- 5 (b) under physiological conditions.

17. A composition as in claim 15 wherein said oligonucleotide is selected from the group consisting of

- (a) oligonucleotides comprising at least 20 consecutive bases from SEQ ID NO.: 1;
 - 10 (b) oligonucleotides comprising at least 20 consecutive bases from the joined exons of SEQ ID NO.: 1; and
 - (c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or
- (b) under physiological conditions.

15 18. A composition as in claim 15 wherein said oligonucleotide is selected from the group consisting of

- (a) oligonucleotides comprising at least 10 consecutive bases from SEQ ID NO.: 2;
 - (b) oligonucleotides comprising at least 10 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 2; and
 - 20 (c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or
- (b) under physiological conditions.

19. A composition as in claim 15 wherein said oligonucleotide is selected from the group consisting of

- 25 (a) oligonucleotides comprising at least 20 consecutive bases from SEQ ID NO.: 2;
 - (b) oligonucleotides comprising at least 20 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 2; and
 - (c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or
- (b) under physiological conditions.

30

20. A composition as in claim 15 wherein said oligonucleotide comprises a nucleotide

sequence selected from the group consisting of SEQ ID NO.: 5, SEQ ID NO.: 6, SEQ ID NO.: 7, SEQ ID NO.: 8, and SEQ ID NO.: 9.

21. A composition as in claim 15 wherein said oligonucleotide is a modified
5 oligonucleotide.
22. A composition as in claim 15 wherein said oligonucleotide is a modified oligonucleotide including at least one synthetic internucleoside linkage.
- 10 23. A composition as in claim 22 wherein said synthetic internucleoside linkage is selected from the group consisting of phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamides, and carboxymethyl esters.
- 15 24. A composition as in claim 21 wherein said oligonucleotide is a modified oligonucleotide having at least one low molecular weight organic group covalently bound to a phosphate group of said oligonucleotide.
25. A composition as in claim 21 wherein said oligonucleotide is a modified
20 oligonucleotide having at least one low molecular weight organic group covalently bound to a 2' position of a ribose of said oligonucleotide.
26. A composition as in claim 21 wherein said oligonucleotide is a modified oligonucleotide having covalently attached thereto a compound selected from the group
25 consisting of androgen, androgen derivatives, estrogen, estrogen derivatives, estramustine, emcyt and estracyt.
27. A pharmaceutical kit comprising the pharmaceutical composition of claim 15 in a pharmaceutically acceptable carrier for intravenous administration.
- 30 28. A method for treating a patient diagnosed as having benign prostatic hyperplasia

or a prostatic cancer comprising

administering to said patient a therapeutically effective amount of a composition comprising an antisense oligonucleotide which selectively hybridizes to a gene or mRNA sequence of said patient;

- 5 wherein said antisense inhibits expression of said gene or mRNA sequence; and
 wherein said antisense inhibits or represses prostatic cell growth.

29. A method as in claim 28 wherein said gene is selected from the group consisting of a PSA gene, a probasin gene, an α FGF gene, an androgen receptor gene, an estrogen receptor
10 gene, a telomerase gene, a prohibitin gene, a src gene, a ras gene, a myc gene, a blc-2 gene, a protein kinase-A gene, a plasminogen activator urokinase gene and a methyl transferase gene.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/15081

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 C07H21/04 A61K31/70 //A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07H A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 05268 A (BAYLOR COLLEGE MEDICINE) 17 March 1994 see page 8, line 14 - page 10, line 20 see example 1 see claims 1,2,17-21,32-35 ---	1,7,13, 15,21, 28,29
X	WO 89 09791 A (UNIV NORTH CAROLINA) 19 October 1989 see page 2, line 12 - line 32 see page 24 ---	1,28,29
X	WO 95 11301 A (UNIV MICHIGAN) 27 April 1995 see claims --- -/--	28,29

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

14 February 1997

Date of mailing of the international search report

26.02.97

Name and mailing address of the ISA

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Fax (+31-70) 340-3016

Authorized officer

Andres, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/15081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER RESEARCH, (1994 MAY 1) 54 (9) 2372-7., XP002025258 ACHBAROU, A. ET AL.: "Urokinase overproduction results in increased skeletal metastasis by prostate cancer cells in vivo." see the whole document ---	28,29
A	CANCER SURVEYS, vol. 11, 1991, pages 239-254, XP000616360 SHERIDAN, V. & TEW, K.: "Mechanism based chemotherapy for prostate cancer" cited in the application see the whole document ---	12,26
O,A	ANTISENSE RES.DEV. 5 (FALL 1995); PAGE 239; ABSTRACT III12, XP002025259 HEAD, M. ET AL.: "Penetration and stability of antisense oligonucleotides injected into the early embryonic chick eye" see abstract & INT.CONF.: 'THERAPEUTIC OLIGONUCLEOTIDES FROM CELL TO MAN'; 4 TO 7 APRIL 1995; SEILLAC; FRANCE, ---	1,4-9
P,X	US 5 556 956 A (ROY ARUN K ET AL) 17 September 1996 see the whole document ---	1,7-10, 13,15, 21-24, 27-29
P,X	CELL GROWTH AND DIFFERENTIATION, (1996 MAY) 7 (5) 573-86., XP000616505 SHAIN, S. ET AL.: "Endogenous fibroblast growth factor - 1 or fibroblast growth factor -2 modulate prostate cancer cell proliferation." see the whole document ---	1,4-9, 28,29
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 MAY 31) 271 (22) 13228-33., XP002025260 BOFFA, L. ET AL.: "Invasion of the CAG triplet repeats by a complementary peptide nucleic acid inhibits transcription of the androgen receptor and TATA-binding protein genes and correlates with refolding of an active nucleosome containing a unique AR gene sequence." see the whole document ---	1-3,7,8, 10,28,29
1		
3	P,X	WO 96 03875 A (UNIV EMORY) 15 February 1996
	P,A	see page 11, line 12 - page 13, line 21 ---
		-/--

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 96/15081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,P, X	<p>PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 37 (0), March 1996, page 344 XP002025261 STEINER, M. ET AL.: "Gene therapy of advanced prostate cancer by in vivo transduction with prostate-targeted antisense c- myc RNA retroviruses." see abstract #2349 & 87TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, WASHINGTON, D.C., USA, APRIL 20-24, 1996., -----</p>	28,29

INTERNATIONAL SEARCH REPORT

Intern: al application No.

PCT/US 96/ 15081

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 96/ 15081

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claims 1-14, 28-29 (as far as in vivo methods are concerned) are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Info. on patent family members

International application No

PCT/US 96/15081

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9405268	17-03-94	AU-A- 4846793	29-03-94
WO-A-8909791	19-10-89	EP-A- 0365657	02-05-90
WO-A-9511301	27-04-95	AU-A- 7983294	08-05-95
US-A-5556956	17-09-96	NONE	
WO-A-9603875	15-02-96	AU-A- 3071995	04-03-96